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INFLUENCE OF FORMALDEHYDE ON THE DIGESTIVE ENZYMES.

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INFLUENCE OF FORMALDEHYDE ON THE DIGESTIVE ENZYMES.

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This study of the influence of formaldehyde on the digestive enzymes was undertaken with a view of determining for formaldehyde the strength of solutions necessary to cause interference in the action of rennet, pepsin, pancreatin, steapsin, ptyalin, amylopsin, and galactase, in vitro, and what strength would at the same time prevent bacterial development.

The work was done with milk, because milk is, of all foods, the one which most readily undergoes decomposition, and consequently is extensively treated with preservatives, and also because milk is a food on which the activity of most of the digestive enzymes may be determined.

Fresh extracts of the various glands from healthy animals were used to furnish the enzymes which could thus be obtained.

Experiment 1.—Action of formaldehyde on calf's rennet.

The rennet was obtained from the mucous membrane of a calf's stomach by digesting it for twenty-four hours at room temperature in 200 c. c. of a 0.2 per cent hydrochloric-acid solution, filtered, and carefully neutralized. One cubic centimeter of neutralized liquid was used to coagulate 10 c. c. of milk. Formaline[a] was added to 100 c. c. of fresh milk in flasks in the following proportions:

TABLE I.—*Showing strength of formaldehyde solution added.*

Flask No.—	Formaline.	Formaldehyde equivalent.	Flask No.—	Formaline.	Formaldehyde equivalent.
	Ratio.	*Ratio.*		*Ratio.*	*Ratio.*
1.....	Control.	.....	7.....	1:750	1:1,875
2.....	1:20	1:50	8.....	1:1,000	1:2,500
3.....	1:50	1:125	9.....	1:1,500	1:3,750
4.....	1:100	1:250	10....	1:2,000	1:5,000
5.....	1:200	1:500	11....	1:3,000	1:7,500
6.....	1:500	1:1,250	12....	1:5,000	1:12,500

The flasks containing the milk and formaldehyde were corked and set aside at room temperature. Fresh milk was always used in the control. At various intervals 10 c. c. of milk was taken out of each

[a] Formaline is a 40 per cent solution of formaldehyde gas in water.

flask and placed in test tubes, 1 c. c. of rennet solution added, and the tubes placed in the incubator at 40° C.

TABLE II.—*Showing the results of Experiment 1.*

From flask No.—	Proportions of formaldehyde.	Mixtures of milk and formaldehyde tested.		
		After 10 minutes.	After 24 hours.	After 48 hours.
1.....	Control.	Solid in 7 minutes	Solid in 7 minutes	Solid in 7 minutes.
2.....	1:50	Not coagulated in 18 hours.	Not coagulated in 18 hours.	Not coagulated in 18 hours.
3.....	1:125	.....do.....	.....do.....	Do.
4.....	1:250	.....do.....	.....do.....	Do.
5.....	1:500	.....do.....	.....do.....	Do.
6.....	1:1,250	Solid in 10 minutes	Solid in 10 minutes	Solid in 10 minutes.
7.....	1:1,875	.....do.....	.....do.....	Do.
8.....	1:2,500	Solid in 7 minutes	Solid in 7 minutes	Solid in 7 minutes.
9.....	1:3,750	.....do.....	.....do.....	Do.
10.....	1:5,000	.....do.....	.....do.....	Do.
11.....	1:7,500	.....do.....	.....do.....	Do.
12.....	1:12,500	.....do.....	.....do.....	Do.

These results show that formaldehyde added to milk in the proportion of 1:2,500 does not interfere with the rennet's coagulation, while the proportion of 1:1,875 retards the coagulation, and the proportion of 1:500 renders the milk incapable of being coagulated in eighteen hours.

Experiment 2.—Action of formaldehyde on pepsin.

The pepsin used was in the form of artificial gastric juice prepared from the stomach of a pig. The stomach was opened, emptied of the contents, and then the surface cleaned with a wet sponge. The mucous membrane was removed from all but the pyloric end of the organ; it was then freed from a portion of the water which was adhering to it by pressure between dry cloths and minced. The finely divided mucous membrane was then placed in 2 liters of dilute hydrochloric acid, containing 6 c. c. of concentrated hydrochloric acid per liter, and the mixture digested in the incubator at 40° C. for a day. The liquid was then filtered through paper, and 50 c. c. added to 200 c. c. of a 0.1 per cent hydrochloric acid solution; 10 c. c. of this solution was added to 1 c. c. of milk treated as in Table I and set aside in incubator at 40° C.

It has been demonstrated by Hammarsten and other workers[1-2] that milk, when subjected to the action of pepsin hydrochloric acid, is broken up into calcium paracasein and a small amount of albumose-like albumin. The paracasein is precipitated, and may be recognized after digesting the milk for a short time, the paracasein separating and finally decomposing with the formation of paranuclein. Any foreign matter that interferes with the formation of paranuclein interferes with the digestion of the milk. This feature was taken to determine when the formaldehyde affected the digestibility of the milk. It was found that upon the addition of formaldehyde in the proportion

of 1:50 the pepsin digestion was retarded, while in a stronger solution, or in the proportion of 1:25, the digestion was materially interfered with; and in the proportion of 1:125 or less, the digestion was normal with the control.

Experiment 3.—Action of formaldehyde on pancreatin.

Two hundred grams of finely chopped fresh pancreas, free from fat was digested in a liter of water for twenty-four hours, filtered, and the solution made up to twice its volume with a 2 per cent solution of sodium carbonate; 10 c. c. of this mixture was added to 1 c. c. of milk, as in Experiment 2, placed in the incubator at 40° C. and tested every two hours until the control gave no precipitate when saturated with magnesium sulphate, thus showing that all the proteids were in the form of pepton. At the end of eighteen hours the control showed the absence of all proteids that were precipitated by magnesium sulphates, and the tubes containing formaldehyde in the proportion of 1:2,000 or less showed the same, while in the tubes containing formaldehyde in the proportion of 1:1,500 or more the digestion was interfered with.

Experiment 4.—Action of formaldehyde on steapsin.

One hundred grams of finely chopped pancreas free from fat was mixed with 1 liter of a solution of glycerine containing 900 c. c. of glycerine and 100 c. c. of a 1 per cent sodium carbonate solution. This mixture was allowed to stand at room temperature for three days, filtered through linen, and the filtrate used for its fat-splitting enzyme; 2 c. c. of this extract was added to 1 gram of neutral fat obtained from cream by shaking with alkaline ether and then washing well with water. To this neutral fat solution were added varying proportions of formaldehyde. To the tubes containing 1 gram of the fat and 2 c. c. of the extract 10 c. c. of water was added, the tubes shaken in a milk shaker, set aside at 40° C., and then shaken every half hour for twelve hours at this temperature. Their acidity was then determined, using phenolphthalein as indicator. It was found that formaldehyde in the proportion of 1:35 prevented the action of steapsin, and in the proportion of 1:50 the action of steapsin was retarded, while weaker solutions had no effect on the activity of the enzyme.

The enzymes used in the preceding experiments practically are the ones whose activity, if interfered with, would affect the digestibility of milk, but formaldehyde is often used as a preservative for food containing a large amount of starch. The enzymes, ptyalin and amylopsin, were studied with regard to their activity with starch when the starch was in the presence of formaldehyde solutions of varying strengths.

Experiment 5.—Action of formaldehyde on ptyalin.

The filtered saliva was used for the ptyalin enzyme. One gram of potato starch, which had been thoroughly washed with dilute hydro-

chloric acid and caustic potash and then washed well with water, was thoroughly mixed in a mortar with cold water, and the thick liquid then poured with constant stirring into boiling water, and the boiling continued about three minutes; 10 c. c. of this mixture was treated with solutions of formaldehyde of varying strength, as shown in the table below, and 2 c. c. of saliva added, the mixture placed in the incubator at 40° C., and tested with iodine solution every two minutes, with the following results:

TABLE III.—*Showing the results of Experiment 5.*

Flask No.—	Proportion of formaldehyde.	Results.
1	Control.	Colorless with iodine after 10 minutes.
2	1:100	Blue with iodine after 10 minutes.
3	1:200	Do.
4	1:500	Do.
5	1:750	Nearly colorless with iodine after 10 minutes.
6	1:1,000	Do.
7	1:1,250	Do.
8	1:1,500	Colorless with iodine after 10 minutes.
9	1:2,000	Do.
10	1:5,000	Do.
11	1:10,000	Do.

The result of these experiments show that the ptyalin's action on starch is interfered with only after formaldehyde has been added to the starch in the proportion of 1:1,250, while in 1:1,500 or less, the ptyalin's activity is normal.

Experiment 6.—Action of formaldehyde on amylopsin.

One hundred grams of finely chopped pancreas free from fat were digested five days, with an occasional shaking, with 400 grams of 25 per cent alcohol, filtered, and 10 c. c. added to 100 c. c. of starch paste prepared as in Experiment 5, with the following results:

TABLE IV.—*Showing the results of Experiment 6.*

Flask No.—	Proportion of formaldehyde.	Results.
1.....	Control.	Colorless with iodine after 25 minutes.
2.....	1:200	Blue with iodine after 25 minutes.
3.....	1:500	Do.
4.....	1:750	Nearly colorless with iodine after 25 minutes.
5.....	1:1,000	Colorless with iodine after 25 minutes.
6.....	1:1,250	Do.
7.....	1:1,500	Do.
8.....	1:2,000	Do.
9.....	1:5,000	Do.

These results show that when formaldehyde was added to starch in the proportion of 1:500 it interfered with the action of the amylopsin, while in the proportion of 1:1,000 or less it had no marked effect.

Summarizing the results of these experiments *in vitro*, we find that formaldehyde may be added to foodstuffs in the proportion of 1:2,500 without affecting the activity of any of the enzymes used. However, there is present in milk an active proteolytic enzyme, galactase, discovered by Babcock and Russell[3] and found by them to play an important part in the ripening of cheese. The part this enzyme plays in the digestion of the milk when taken into the system has not as yet been entirely proven. Snyder[4] studied the effect of the enzymes in milk, and claims that when milk was used in a mixed diet the protein was from 4 to 5 per cent more digestible than when the milk was omitted. Snyder[4] also claims that, upon digesting toast for two hours with 10 c. c. of milk, 12.20 per cent of the protein of the toast was digested. These results suggested that the soluble or chemical ferments of milk were the active agents which caused this increase in digestibility. Very little work has been done to show the effect of formaldehyde on the activity of the enzyme galactase. Babcock and Russell[5] claim that formaldehyde prevents the action of galactase, although they quote no experiments to prove their assertion. Van Slyke[6] concluded, from experiments conducted at the New York Experiment Station, that formaldehyde in 0.1 per cent solution inhibits the action of this enzyme. No weaker solution of the formaldehyde was tried.

In studying the effect of formaldehyde on the enzyme galactase, the concentrated extract of the enzyme was used. This was obtained by allowing the slime that was taken from the milk-separator cylinder to macerate for forty-eight hours in a closed vessel containing water, to which chloroform had been added in excess. This emulsion was filtered, and 25 c. c. of the filtrate added to each of the seven sterile flasks. Formaldehyde was then added to Flasks Nos. 1, 2, 3, and 4 in the proportion of 1:1,500, 1:2,500, 1:5,000, and 1:10,000, respectively, while Flasks Nos. 5, 6, and 7 were not treated. The flasks were set aside at room temperature, and at the end of forty-eight hours to Flasks Nos. 1, 2, 3, 4, and 5 was added 100 c. c. of milk collected under antiseptic conditions and containing 20 per cent chloroform. To Flasks 6 and 7 was added 100 c. c. of milk collected under the same conditions but containing formaldehyde in the proportions of 1:1,000 and 1:2,000, respectively. Flask No. 5 served as a control, Babcock[5] having shown that chloroform, when added to milk even as high as 30 per cent, had no effect on the activity of the enzyme galactase. Plates were made, using 1 c. c. of the content of each flask, and showed the absence of bacteria. The total nitrogen and soluble nitrogen were then determined in 10 c. c. of the mixtures, the flasks set aside at 40° C. for two weeks, and at the end of this time plates were again made, and showed that the mixtures were still free from bacteria. The total nitrogen and soluble nitrogen were also

determined, as before. The results are summarized in the following table:

TABLE V.—*Showing the effect of formaldehyde on galactase.*

Flask No.—	Extract and formaldehyde.	Milk and—		Total nitrogen at beginning of experiment.	Soluble nitrogen at beginning of experiment.	Total nitrogen at end of experiment.	Soluble nitrogen at end of experiment.
		Chloroform.	Formaldehyde.				
	Ratio.	*Per cent.*	*Ratio.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1.....	1:1,500	20	.....	0.56	0.11	0.57	0.15
2.....	1:2,500	20	.....	.51	.10	.51	.13
3.....	1:5,000	20	.....	.54	.10	.56	.18
4.....	1:10,000	20	.....	.53	.10	.56	.24
5.....	.....	20	.....	.53	.10	.56	.26
6.....	.....	.....	1:1,000	.55	.09	.56	.09
7.....	.....	.....	1:2,000	.57	.10	.58	.10

These results show that formaldehyde retards the action of the enzyme galactase when used in very concentrated solutions, but in weaker solutions it seems to have little effect, and, although the minimum solution necessary for preserving purposes was not tried, the results from the solutions that were used go to show that the formaldehyde when used in milk in sufficient amount to preserve the milk would have no material effect on the enzyme.

As Snyder[4] claims that 12.2 per cent of the protein in toast was digested in two hours by milk alone, I attempted to determine the amount of protein in toast that was digested in two hours by the extract of the enzyme prepared as in the previous experiment, and also with the milk alone, and then to study the effect of solutions of formaldehyde of varying strengths on the digestive properties of the enzymes, with the following results:

TABLE VI.—*Showing the effect of formaldehyde on the digestive properties of galactase.*

	Per cent.
Soluble nitrogen before digestion:	
10 grams toast, 10 c. c. extract, chloroform and 90 c. c. water..	2.22
Soluble nitrogen after digestion:	
10 grams toast, 10 c. c. extract, chloroform, no formaldehyde.	2.22
10 grams toast, 10 c. c. extract, chloroform, 1:2,500 formaldehyde	2.28
10 grams toast, 10 c. c. extract, chloroform, 1:5,000 formaldehyde	2.22
10 grams toast, 10 c. c. extract, chloroform, 1:10,000 formaldehyde	2.28
10 grams toast, 10 c. c. extract, chloroform, 1:20,000 formaldehyde	2.28
Soluble nitrogen before digestion:	
10 grams toast, 10 c. c. milk, chloroform and 90 c. c. water	2.34
Soluble nitrogen after digestion:	
10 grams toast, 10 c. c. milk, chloroform, no formaldehyde ...	2.34
10 grams toast, 10 c. c. milk, chloroform, 1:2,500 formaldehyde.	2.36
10 grams toast, 10 c. c. milk, chloroform, 1:5,000 formaldehyde.	2.34
10 grams toast, 10 c. c. milk, chloroform, 1:10,000 formaldehyde.	2.34
10 grams toast, 10 c. c. milk, chloroform, 1:20,000 formaldehyde.	2.34

These results are contrary to those of Snyder.[1] As will be noticed, the amount of soluble nitrogen after two hours' digestion is the same as the amount previous to digestion. This difference in results is probably due to my using an antiseptic, while in Snyder's work, no antiseptic being used, bacteria are responsible for the increase in soluble nitrogen and not the enzyme galactase.[a]

Formaldehyde having been found to be harmless in its effect on the activity of these enzymes when used in solutions of such strength as are necessary for preserving food material and there being often danger of development in foodstuffs of various organisms which may be taken into the system through the food and liberate toxins which produce serious trouble, it was of interest to determine whether the formaldehyde would arrest development or kill the organism when it came in contact with it in the foodstuffs.

Some of the organisms commonly found in milk, such as *Bacillus acidi lactici*, *B. subtilis*, *B. typhosus*, *B. coli communis*, and *Staphylococcus pyogenes aureus*, were inoculated into bouillon tubes containing formaldehyde of different proportionate strengths and set aside in the incubator for twenty-four hours. Formaldehyde in the proportion of 1:20,000 prohibited development under these conditions. This, of course, does not show that formaldehyde killed the organisms. This point was also determined by inoculating another set of tubes with the organisms and allowing them to develop for twenty-four hours. Then formaldehyde solutions of varying strength were added and the tubes set aside in the incubator. At the end of twenty-four, forty-eight, and seventy-two hours, 1 c. c. was taken from each tube and added to flasks containing 50 c. c. of fresh bouillon solutions, these flasks set aside in the incubator, and the results noted. These results showed that it required formaldehyde solutions in the proportion of 1:1,560 to destroy the organisms in twenty-four hours, while the proportion of 1:1,870 destroyed the organisms in seventy-two hours. It was found that the organisms varied in their resisting power, but the strength solutions given include all these organisms.

We find a great number of people who hold that artificial digestion experiments and animal digestion experiments are worthless. It is true that a majority of conductors of artificial digestion experiments used commercial products for their active enzymes instead of fresh extracts of the various glands, and some of these workers have made no attempt to duplicate the mechanical conditions which obtain in the living stomach. The general value of their conclusions is somewhat lessened on this account, but the results, being in most cases comparative, certainly can not be said to be worthless. The results of the

[a] Snyder has recently duplicated his work, using an antiseptic, and quotes results in Bul. No. 86 of the Minnesota Experiment Station concordant with those he obtained when no antiseptic was used.

majority of workers who have experimented with formaldehyde are of no practical value, as we find on reviewing the literature on this subject that most workers[7-13] have used formaldehyde solutions varying in proportion from 1:25 to 1:2,000, which is out of proportion to the amount actually used or required. The chief object of most of the work that has been done was to see if formaldehyde interfered with digestion. In most cases it appears as if the workers took quantities of formaldehyde large enough to insure interference and made no attempt to study the minimum amount that could be used without interference.

CONCLUSIONS.

From the experiments the following may be drawn:

(1) Formaldehyde added to milk in the proportion of 1:20,000 preserved the milk for forty-eight hours.

(2) Formaldehyde added to milk in the proportion of 1:2,500 or less has no effect on the activity of the fresh enzymes, rennet, pepsin, pancreatin, and steapsin *in vitro*.

(3) Formaldehyde added to starch in the proportion of 1:2,500, or less, has no effect on the conversion of the starch by the enzymes, ptyalin, and amylopsin *in vitro*.

(4) Formaldehyde added to milk in sufficient quantity to preserve the milk for forty-eight hours—that is, 1:20,000—does not interfere with the action of the enzyme galactase *in vitro*.

(5) Formaldehyde added to milk in the proportion of 1:20,000 prevents the development of the more common bacteria found in milk, and when added in the proportion of 1:1,560 it kills these bacteria.

(6) Formaldehyde may be added to milk in sufficient quantities to preserve the milk and prevent the development of some of the more common bacteria—that is, 1:10,000—and still have no deleterious effect on the digestibility of the milk *in vitro*.

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